A DISSERTATION

ON

ESTROGEN RECEPTOR STATUS IN UPPER GASTROINTESTINAL MALIGNANCIES

Submitted for

M. S. GENERAL SURGERY
BRANCH – I
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KILPAUK MEDICAL COLLEGE,
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CERTIFICATE

This is to certify **Dr.S.SENTHIL KUMAR,** a bonafide MS General Surgery Post Graduate Student, from Government Royapettah Hospital, Kilpauk Medical College, Chennai – 600 010 has submitted the dissertation on **ESTROGEN RECEPTOR STATUS IN UPPER GASTROINTESTINAL MALIGNANCIES** in partial fulfilment of the requirements for M.S. General Surgery (Branch – I) Degree Examination of **THE TAMIL NADU Dr.M.G.R. MEDICAL UNIVERSITY, GUINDY, CHENNAI,** to be held in September 2006.

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CONTENTS

PREFACE

Gastric cancer is a disease with poor prognosis. The incidence of esophageal cancer is increasing throughout the world in both sexes and it also carries a very poor prognosis. There is still much to understand about the genes that contribute to the progression of above malignancies..

The activated estrogen receptor gene mediates inhibition of cell division, suppress growth of genetically deranged cells and also effectively acts as a tumour suppressor gene, in carcinogenesis. In upper gastrointestinal malignancies, the role of the Hormonal receptor, estrogen receptor is still under evaluation.

The identification of estrogen receptor is not only an important predictive test for the endocrine manipulation of the tumour, but also an essential target for the drug action. Control of estrogen receptor in breast cancer is well established now. A detail trial on endocrine manipulation of upper gastro intestinal malignancies, is not available so far. For this, detection of estrogen receptor is mandatory. This study addresses the above need.

INTRODUCTION

Hormones are important regulators of growth. By stimulating proliferation, hormones may increase the risk of mutation and at the same time stimulate the replication of mutated cell. Thus Hormones are complete carcinogens. An excellent example is Enterochromaffin like cell carcinoid of the stomach, which is caused by hypergastrinaemia, and where pathogenesis is diffuse hyperplasia, linear and nodular hyperplasia, dysplasia, intramucosal carcinoid and invasive carcinoid (11).

Beatson's original observation on breast cancer regression after ovariectomy published in 1896, provided the first insight into the hormone dependent nature of the tumours. In 1960, Jensen et al discovered the existence of estrogen receptor (ER) in the cytoplasm of human mammary cancer cells. In 1961, folca and others monitored in vivo uptake of synthetic radiolabelled estrogen, in breast tumors by administering tritiated hexestrol, to women about to undergo mastectomy and subsequently found that, women who would respond to endocrine ablation had a greater uptake of radioactive hexestrol in their neoplasm than did non responding patients (2). Numerous studies have subsequently shown that approximately half of all biopsy specimen of malignant breast tumours contain estrogen receptor (3,4,5).

Jensen and others proposed the estrogen receptor assay to predict responsiveness to endocrine therapy and this was subsequently refined by also measuring the progesterone receptor (6,7). The oncologist's use of these biochemical criteria (Presence of estrogen receptor and progesterone receptor) has increased by twofold or three fold, the accuracy of selecting the patients with breast cancer who are most likely to respond objectively to endocrine manipulation (4,5). Estrogen receptor positivity and their positive impact on the management of hormone dependent tumours like breast, ovary, uterine, prostatic cancer is well established only very few papers have been published about the estrogen receptor positivity in non-hormone dependent tumours like liver, stomach, pancreas, rectum and lung.

In 1983, Tokunga et al first reported estrogen receptor expression in gastric cancer (10) but it role in gastric carcinogenesis remained unknown. Since then estrogen receptors are reported to be present in malignancies of non reproductive organs like liver (12) stomach (13,14), pancreas (15) Rectum (14,16) and lung.

There are very few publications about the estrogen receptors status in upper gastrointestinal malignancies. Cited publications proved the presence of estrogen receptors in esophageal carcinoma cell line (17) and

gastric carcinoma. (10,19). They also proved that 17 ß, estradiol has growth inhibitory effect on estrogen receptor positive esophageal cancer cell line (18) and estrogen increases apoptosis in human gastric cancer cells (20). Almost all the publications on estrogen receptor in upper gastrointestinal malignancies are from Japan and few from Western Countries that too mostly proven in animal models. To the best of our knowledge, this is the first study on estrogen receptor status in upper gastrointestinal malignancies in this part of the world.

Worldwide gastric cancer in the fourth most common cancer and second leading cause of cancer death (22,28). In India, Gastric cancer is the most common cancer among all and the most common cause of cancer death. Worldwide esophageal cancer ranks fifth in the mortality rate among tumour sites (29). In India, esophageal cancer is the 3rd most common cancer in men and 5th most common cancer in women.

Recognition of estrogen receptor status in upper gastro intestinal malignancies will help us using hormonal therapy, even in situations where other modalities of treatment fail. Existing studies reveal that the estrogen receptor positivity in upper gastro intestinal malignancies range from 0-65%, depending on the method of study.

In this study, we experimentally identified the estrogen receptor positivity in upper gastrointestinal malignancies, which are the most common and leading killers among GI malignancies in India we also analysed and compared our study results with international studies.

ESTROGEN RECEPTOR

In 1960, Jensen *et al.,* first proposed estrogen receptor, based on pioneering studies in immature rats, that proved radiolabelled estradiol would bind preferentially in estrogen target tissues such as uterus, vagina and pituitary gland. The rat uterine estrogen receptor was subsequently isolated and shown to be an extractable estrogen binding protein (30,31). The estrogen receptor is a nuclear transcription factor, that is a member of the steroid receptor superfamily (32).

Recent studies have revealed the existence of two distinct estrogen receptors in our body, estrogen receptor α and estrogen receptor ß. The genes for both receptors are encoded by 8 Exons, which are located on different chromosomes. The gene for estrogen receptor α found on the long arm of chromosome 6 (6q 25.1) and for estrogen receptor ß on chromosome 14 (33).

While both estrogen receptor α and estrogen receptor ß bind estrogen as well as other agonists and antagonists, the two receptors have distinctly different localisation and concentration within our body. Estrogen receptor α is found in the liver, estrogen receptor ß in gastrointestinal tract and both receptors are distributed in cns, mammary gland, cardiovascular

system, urogenital tract and bone (34). The first complete human estrogen receptor ßcDNA was cloned and found to contain 530 aminoacids (35).

Two of the most interesting sites on the estrogen receptor molecule are its ligand binding domain (LBD) otherwise known as AF-2, and growth factor binding domain, otherwise known as AF-1. In addition, the DNA binding domain (DBD) is responsible for binding at estrogen response elements (ERE) on the chromosome.

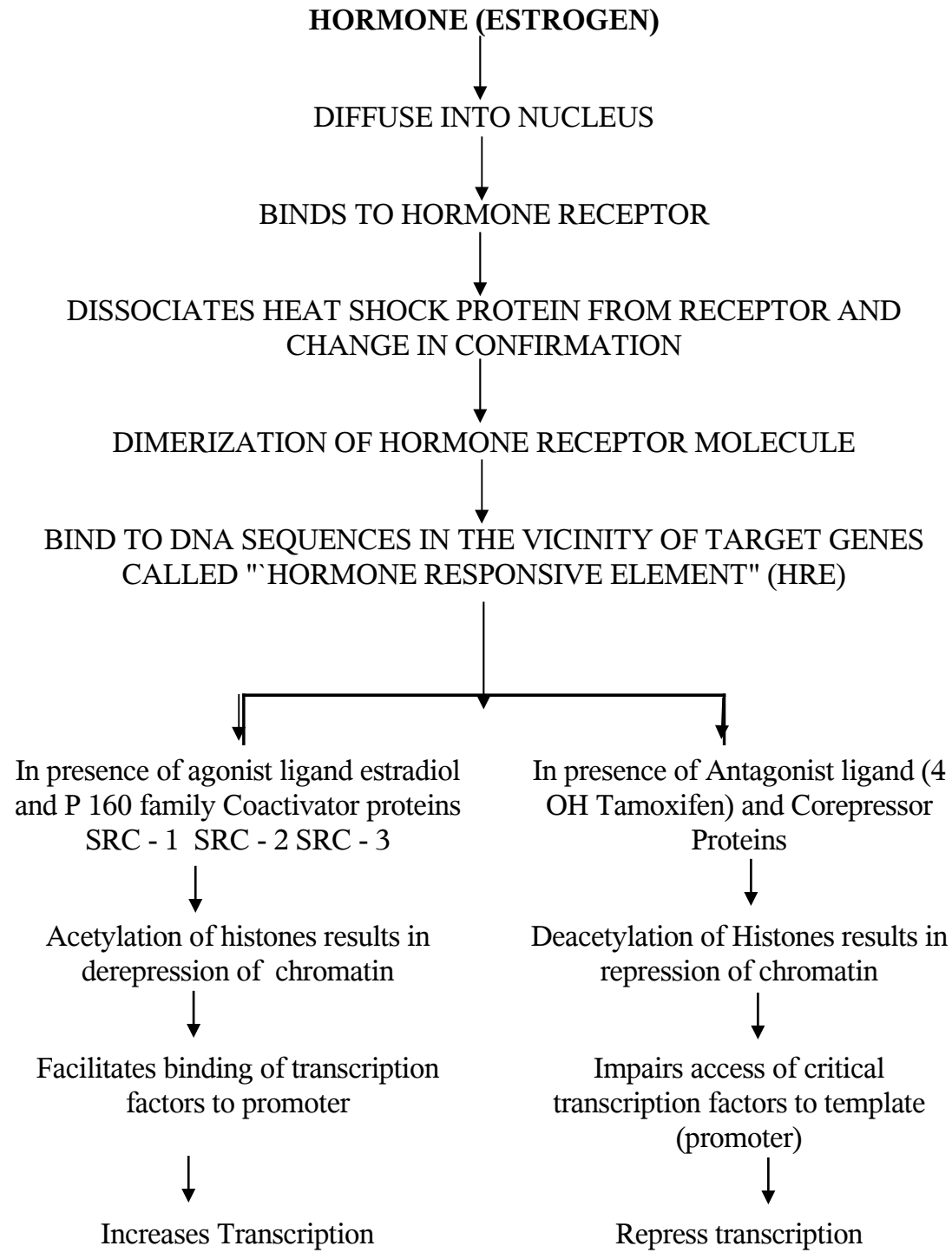
A subtle difference between the two receptors is in their ligand - binding pockets, in the substitution of Leu 338 in estrogen receptor α with met 384 in estrogen receptor ß (34).

In one study on immunolocalisation of estrogen receptor α and ß in gastric epithelium and enteric neurons, Estrogen receptor α and ß proteins were detected in the nuclei of fundic parietal cells and epithelial cells in the progenitor zone. In the antrum, several cells are immunoreactive for estrogen receptor ß, in the region containing stem cells and neuroendocrine cells but estrogen receptor α protein was not detected.

Both estrogen receptor α and ß proteins were expressed in enteric neurons within the nucleus and cytoplasm, with specific punctate staining for estrogen receptor in cell bodies and fibres (36).

FUNCTION

Estrogen interact with their nuclear receptor in target tissue cells to modulate hormone responsive gene expression.



FUNCTIONAL DIFFERENCES

Interestingly, estrogen receptor α and ß when complexed with estrogen, were shown to signal in opposite ways from an AP-1 site, with estrogen activating transcription in the presence of estrogen receptor α and inhibiting transcription in the presence of estrogen receptor ß.

The location of estrogen receptors in gastric epithelium and enteric neurons imply that direct regulation of multiple cell types by estrogens may contribute to the modulation of gastric functions, that have been recognized during the estrous cycle and between sexes (36).

Estrogen increases apoptosis in human gastric cancer cells (20). Recently estrogen has been found to stimulate the expression of trefoil peptides in the stomach (21,46), which play a key role in mucosal protection through mucous barrier formation and in mucosal repair, through promotion of restitution after injury. Estrogen receptor genes may also act as tumour suppressor gene.

Most recent drugs targeted to estrogen receptors such as Tamoxifen, ICI-164384, Raloxifene, act as either estrogen receptor agonist or antagonist depending on species, tissues, and administered dose.

PURPOSE OF STUDY

In a recent study, global age specific pattern of the male to female ratio of gastric cancer suggests basic biological differences between the sexes, (preventive effect of estrogen), that could explain the worldwide male predominance in the incidence of gastric cancer.(22)

In Sweden, three case control studies and one cohort study conducted on postmenopausal women all indicated a negative association between longer fertility and gastric cancer (23,24,25,26). Further in a placebo controlled randomized study on adjuvant treatment of breast cancer, there was an increased risk of gastric cancer among women, who received the drug tamoxifen, used mainly for its antiestrogen effect on estrogen receptor positive breast cancer (37).

Long arm of chromosome 6, containing estrogen receptor α gene is regarded as a site with frequent loss of heterozygosity (LOH) in gastric cancer. Deletion of long arm of chromosome 6, is common is gastric carcinoma (38) suggesting the presence of tumour suppressor genes in this region (45). There are enough publications that estrogen receptor may mediate inhibition of cell division and the activated estrogen receptor gene was reported to suppress growth of a neuroblastoma cell line (39).

Introduction of estrogen receptor gene into estrogen receptor negative colon carcinoma cells were found to cause marked growth suppression (40). From a swedish study, they found that reduced risk of gastric cancer in a male cohort (Prostate cancer patients) exposed to estrogen. Studies also proved that growth inhibition of estrogen receptor positive esophageal cancer cell line, by 17ß estradiol (E2) is mediated by signal transduction induced by estrogen-estrogen receptor system (18).

Before recommending hormonal treatment for upper gastrointestinal malignancies we should know about the estrogen receptor status in India; thereby percentage of patients who would respond well is known. So we experimentally studied about estrogen receptor status with upper gastrointestinal endoscopy specimens by immunohistochemistry method.

LACUNAE IN CURRENT KNOWLEDGE

The two isoforms of estrogen receptors (α and ß), have two different opposite functions. This necessitates us to identify estrogen receptor ß separately which acts as tumour suppressor gene in the absence of estrogen receptor α (47), so that type of hormonal treatment (agonist/ antagonist) to be recommended is known.

Eventhough literature suggest that the effects of estrogen in stomach cancer as well as in normal stomach might be mediated by estrogen receptor ß, the role of estrogen receptor ß might differ by the subtype of stomach adenocarcinoma, specifically signet ring adenocarcinoma. So estrogen receptor status should be studied along with the histopathological subtypes of cancer.

HYPOTHESIS

Abnormalities of the cell cycle are said to be the cause for the initiation and maintainance of malignancy. Normally several key proteins are essential for maintainance of homology. When there is aberrance in expression of any of the key proteins, abnormal cell cycling sets in, malignancy ensues.

Estrogen found to increase apoptosis in human gastric cancer cells and trefoil peptide expression which helps in mucosal protection of stomach. Estrogen also has growth inhibition on estrogen receptor positive esophageal cancer cells.

We have attempted here to identify positivity of estrogen receptor status in esophageal and gastric malignancy, which may pave the way for hormonal manipulation both in therapeutic and preventive aspects in future.

REVIEW OF LITERATURE

- In 1960, Jensen *et al.,* reported for the first time, that after injecting a physiological dose of ^{3}H E_2 into the Hypoderm of a young mouse; the amount of ^{3}H E_2 found in the tissues of uterus, vagina and other parts was greater than that found in blood plasma. This proved for the first time that estrogen receptor protein was present in tissues of uterus and vagina (31).

- In 1961, Folca *et al.,* found that women who would respond to endocrine ablation in breast cancer, had a greater uptake of radioactive hexestrol, a synthetic estrogen compound in their neoplasm (45).

- In 1983, Tokunaga *et al,* first reported estrogen receptor expression in Gastric cancer and its role in gastric carcinogenesis remained unknown (46).

- In 1987, Matsuoka, H. *et al.* established that cell lines from established squamous cell esophegeal carcinoma was found to be moderately responsive to hormones, being inhibited by estrogen and enhanced by testosterone (52).

- In 1988, Yokozaki H. *et al.* Department of Pathology, Hiroshima University, School of Medicine, Japan established that estrogen receptor immunoreactivity positive gastric schirrous carcinomas showed a much worse prognosis than those with estrogen receptor immunoreactivity negative gastric schirrous carcinoma (53).

- In 1989, Utsumi Y, Nakamura T. *et al.* second department of Surgery Shimane Medical University, Izumo, Japan established that inhibitory effect of estrogen on the growth of human esophageal carcinoma cell line was mediated by estrogen receptor (18).

- In 1991, Yasuo Utsumi, M.D. Teruhisa Nakamura M.D. *et al.* established that growth inhibition of estrogen receptor positive esophageal cancer cell line by 17ß estradiol is mediated by signal transduction and induced by the estrogen. estrogen receptor system (18).

- In 1998, H.L. Waldum et al., Department of Medicine and Pathology, University Hospital, and Institute of Physiology and Biomedical Engineering and Morphology, Norwagean University of Science and Technology - Norway, established that hormones by stimulating growth increase the probability of mutation in their target

cell and also they stimulate the growth of mutated cell. Thus hormones are complete carcinogens (45).

- In 1998, Korenja D et al. Department of Surgery, Fukuoka City Hospital, Japan established that sex hormone receptor negative tumours have a higher proliferative activity than sex hormone receptor positive tumor in Human adenocarcinoma of the Gastro intestinal tract (49).

- In 1999, Oshima CT, Wonraht DR et al. Fundacao Oncocentro Sao Paulo, Clinical and Cirurgica Unifesp-Epm Brazil established the presence of estrogen receptor in gastric cancer patients, 50% in males patients, and 75% in female patients and 62.5% in both males and females of adjacent normal gastric tissues by immunohistochemical method.

- In 2001, Campbell Thompson M. Department of Medicine, College of Medicine, University of Florida, gianesville, Florida, USA established that immunolocalization of estrogen receptor alpha and beta in gastric epithelium and enteric neurons and its relations to sexual dimorphism in gastric acid secretion (36).

- In 2002, Matsuyama S et al., Department of Surgery, Saja Medical School, Saja city, Saga, Japan established that estrogen receptor ß was found to be expressed in human gastric adenocarcinoma

including normal gastrointestinal tract. Among signet ring cell adenocarcinomas of the stomach, cytoplasm were stained, in addition to nuclei.

- In 2002, Takano N, Tizuke N, Hazama S. *et al.* Department of Surgery Yamaguchi University School of Medicine, Yamaguchi, Japan established that altered expression of estrogen receptor - alpha and estrogen receptor beta in gastric cancer compared with corresponding normal gastric tissues. They also established altered expression to increased metastatic potential in gastric cancer (42).

- In 2003, Xin Han Zhao *et al.* Department of Oncology, Xijing Hospital, Fourth Military Medical University, Xian Shaanxi Province, China established the expression of estrogen receptor beta in normal gastrointestinal tract. The effects of estrogen in stomach cancer and normal tissues might be mediated by estrogen receptor beta and the role of estrogen receptor - beta might differ by the subtype of stomach adenocarcinoma especially signet ring cell adenocarcinoma. They proved estrogen receptor positive rate of 40% and estrogen receptor mRNA positive rate of 80% in gastric cancer. They also established that estrogen receptor mRNA expression has greater value than estrogen receptor protein expression in clinical application, because of high sensitivity of insitu hybridisation and strong expression in gastric cancer which might be used to judge the

prognosis of tumour and predict the effectiveness of endocrine therapy for gastric cancer (19).

- **In 2004, In Sook Woo *et al.*, Department of Internal Medicine, College of Medicine, Catholic University, Seoul, Korea established that loss of estrogen receptor α expression located at chromosome 6 is associated with hypermethylation near its promotor region ATG start codon in Gastric Cancer cell lines, which leads to gene silencing including tumour suppressor gene (46).**

- **In 2004. Mats Lindblad *et al.,* Department of Surgical Sciences and Pathology and Cytology, Karolinsks University Hospital Stockholm, Sweden established that estrogen prevents gastric cancer in a cohort of men heavily exposed to estrogen (47).**

- **In 2005, Ratna K. Vadlamudi Ph.D., Seetharaman Balasenthil Ph.D. *et al.,* Department of Molecular and Cellular Oncology, the University of Texas, M.D. Anderson Cancer Centre, Houston, Texas, USA established estrogen receptor beta expression in salivary duct adenocarcinoma. They also proved that estrogen receptor ß staining was nuclear and occasionally cytoplasmic in tumour cells. They also established that estrogen receptor ß in absence of estrogen receptor α may play a tumour suppressor function and may provide a novel therapeutic target, using specific agonist in salivary ductal carcinoma (48).**

STUDY DETAILS

Type of Study

Prospective Descriptive Experimental Study.

Study Duration

Jan 1, 2005 to December 31, 2005.

Collaborating Institutions

1. Department of Surgery, Government Royapettah Hospital, Kilpauk Medical College, Chennai - 600 010, India.

2. Department of Surgical Gastroenterology, Government Royapettah Hospital, Kilpauk Medical College, Chennai - 600 010, India.

3. Division of Immuno Histochemistry, R&D Histopath Lab, Mylapore, Chennai - 600 004, India.

DETAILS OF MATERIAL AND EXPERIMENTAL DESIGN

Tissue Specimens

Formalin fixed, paraffin embedded, tissue from endoscopic biopsy of esophageal and gastric malignancy patients were used for this study. Histological sections were studied by the collaborating pathologist at Immuno histochemistry division of the R&D Histopath Lab, Mylapore, Chennai - 4.

Technique of Detection

Immunohistochemistry is a multi step process that requires specialized processing of the tissue, the selection of appropriate reagents and interpretation of the stained tissue sections. In general, immunohistochemistry staining techniques allow for the visualization of antigens, by sequential application of a specific antibody to the antigen, a secondary antibody to the primary antibody which serves as a link between the primary antibody and streptavidin enzyme conjugate, an enzyme conjugate and a chromogenic substrate. The enzymatic activation of the chromogen results in a visible product at the antigen site.

We have used this method in identifying estrogen receptor in esophageal and gastric malignancies, in our study.

MATERIALS AND METHODS

The primary antibody mouse anti-estrogen receptor clone - ER-7G5 was obtained from Zymed Laboratories Inc, South Sanfrancisco, CA 94080, USA. The necessary reagent, buffers and humidifying chambers were utilized from the Immunohistochemistry division R&D Histopath Lab; Mylapore, Chennai-4. The primary monoclonal antibody is generated in Ascitic fluid, protein A purified. The vial is filled to 0.5 ml with reagent containing PBS, 1% BSA, and 0.1% sodium azide.

Tissue section of 5 microns cut with the help of Leica microtome. They were applied to Poly-L-Lysine precoated slides. The following staining protocol was followed. Dewaxing done in xylene bath and sections were brought to water through graded alcohol. They were subjected to microwave antigen retrieval in citrate buffer of pH6 for 30 mins. To block non specific reactivity and staining from endogenous peroxidase, sections were incubated with hydrogen peroxide for 5 minutes.

After rinsing the slides were incubated at room temperature with estrogen receptor primary antibody for 1½ hrs. The slides were washed and biotinylated link was applied and incubated for 30 minutes. The sections were incubated in biotinylated streptavidin HRP for 30 minutes. In between

these stages, the slides were rinsed in 10mM phosphate buffered saline. DAB, a substrate chromogen was applied and the slides were incubated for 5 minutes. The slides were thoroughly rinsed and counterstained with Mayer's Hematoxylin for 30 seconds and then covered with glycerol jelly and cover slip applied.

Throughout the procedure 98 to 100% humidity was maintained in a humid chamber. After the above procedure, the slides were ready for screening.

Immunohistochemistry stained positive cells, look brown and negative cells look blue.

EVALUATION OF STAINING

Intensity of Staining

0 = No staining

1+ = weak, but definitive staining

2+ = Positive

3+ = Strong Positive

(Histopathology, Vol.18, No.6; June 1991).

DATA ANALYSIS

Statistical Methods

The significance of the association between variables was tested by the χ^2 test. The variables included in univariate statistical analysis were gender, age, degree of differentiation, location of the tumour, intensity of staining of estrogen receptor expression levels. All p values reported are for a two-sided test and the level of significance was set at 0.05, SPSS version 14 software was used for the statistical analysis. This was done with help of the statistical analysts, in The Institute of Community Medicine, Madras Medical College, Chennai - 600 003. The basic data collected during endoscopy regarding the age, sex and the location of the tumour and degree of differentiation in the esophagus and stomach were analysed with regards to the staining of estrogen receptor expression obtained after immunostaining.

STATEMENT OF LIMITATIONS

This study is based on, a limited number of patients. A detailed study of more subjects, in relation to histopathology of cancer and lymphnode status and followup for a longer duration will augment the validity of the data.

Many foreign researchers are of the opinion that, compared with other methods in examining estrogen receptor protein, the molecular hybridization in examining estrogen receptor mRNA has a higher sensitivity (41,42) and estrogen receptor mRNA expression has greater value than estrogen receptor protein expression in clinical application. This is because of high sensitivity of insitu hybridization and the strong estrogen receptor mRNA expression in gastric cancer, which can be used to judge the prognosis of tumour and predict the effectiveness of endocrine therapy in gastric cancer.

ETHICAL ISSUES INVOLVED

The study was done in tissue obtained from upper gastrointestinal endoscopy. No ethical conflicts are involved in this study.

ANALYSIS

PATIENT CHARACTERISTICS

n = 50

Characteristics		No. of patients
1. Gender		
Carcinoma Esophagus	=	19
Male	=	8
Female	=	11
Carcinoma Stomach	=	31
Male	=	23
Female	=	8
2. Age		
Carcinoma Esophagus	=	45-75 years
Carcinoma Stomach	=	37-84 years

3. Site

Carcinoma Esophagus = 19

Carcinoma Stomach = 31

4. Histopathology

Squamous Cell Carcinoma = 16

Adenocarcinoma = 34

5. Differentiation

Carcinoma Esophagus

Well differentiated = 8

Moderately differentiated = 6

Poorly differentiated = 5

Carcinoma Stomach

Well differentiated = 16

Moderately differentiated = 11

Poorly differentiated = 4

OBSERVATIONS

AGE BASED OBSERVATION

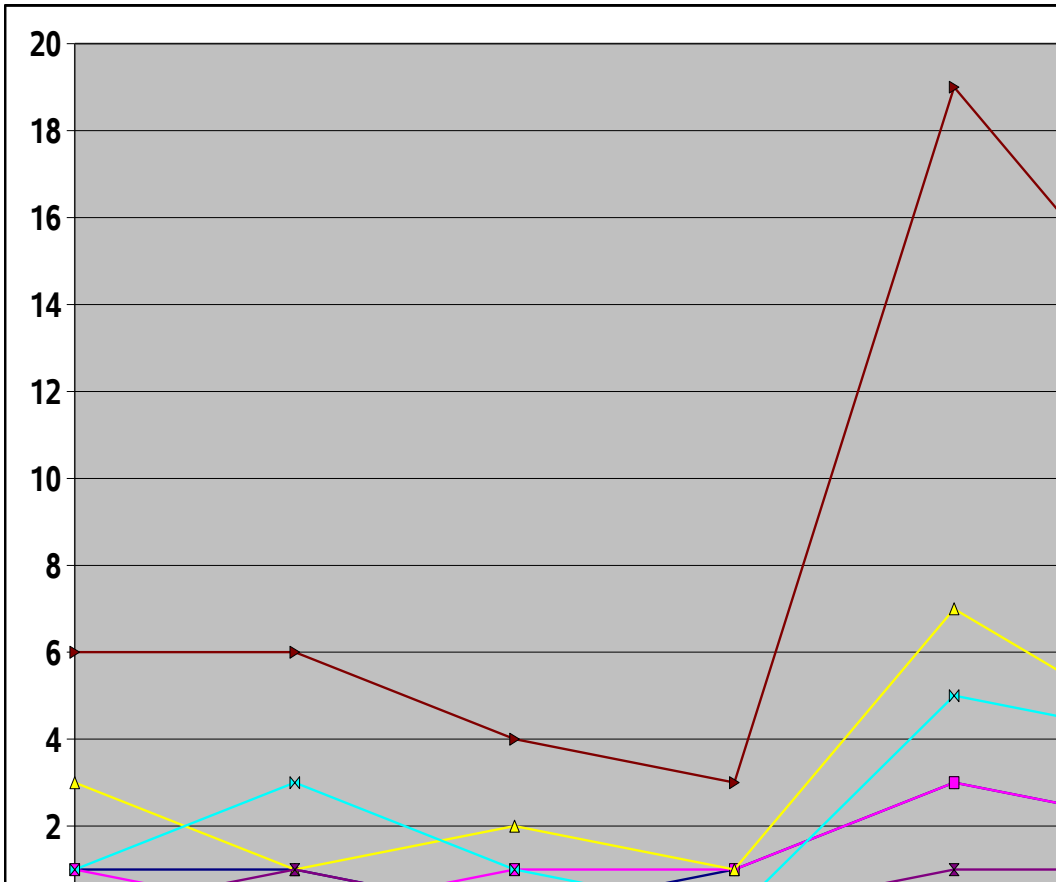
CARCINOMA ESOPHAGUS

Age	Intensity of Staining					
	0	**1+**	**2+**	**3+**	**Total patients**	**Total positivity**
41-50	1	1	0	1	3	2
51-60	1	0	1	1	3	2
61-70	3	1	2	1	7	4
71-80	1	3	1	0	5	4
81-90	0	1	0	0	1	1
Total	6	6	4	3	19	13

The above data was statistically analysed. χ^2 test = 34. 443, df =25, p. value= 0.025, CI = 0.022-0.028. The study patients predominantly belonged to the 7th and 8th decade. The positivity was more appreciated in seventh and eight decade and cent percent estrogen receptor expression seen in 9th decade.

AGE BASED OBSERVATION

CARCINOMA ESOPHAGUS



AGE BASED OBSERVATION

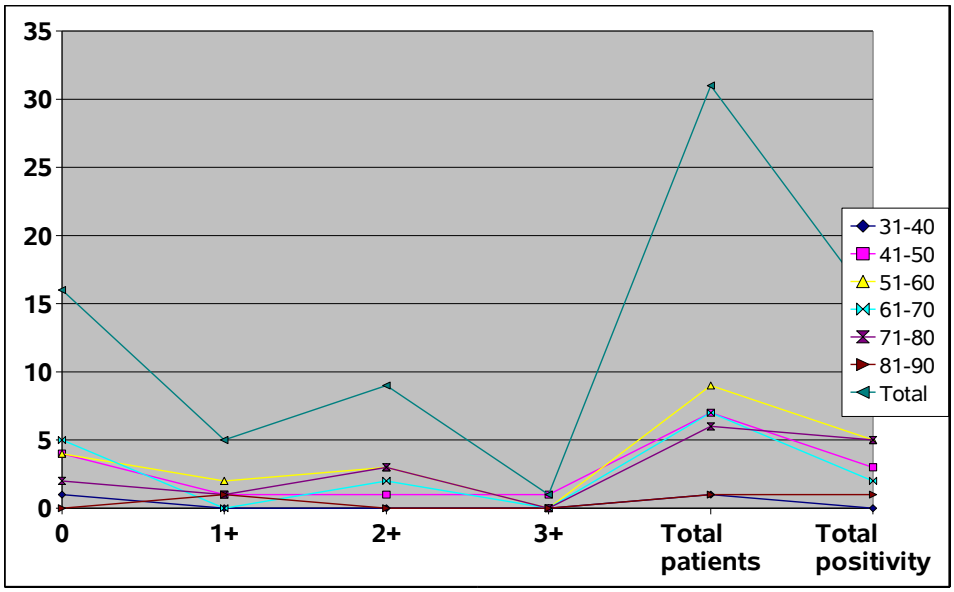
CARCINOMA STOMACH

Age	Intensity of Staining					
	0	**1+**	**2+**	**3+**	**Total patients**	**Total positivity**
31-40	1	0	0	0	1	0
41-50	4	1	1	1	7	3
51-60	4	2	3	0	9	5
61-70	5	0	2	0	7	2
71-80	2	1	3	0	6	4
81-90	0	1	0	0	1	1
Total	16	5	9	1	31	15

The above data was statistically analysed. χ^2 test = 34.443, df =25, p.value= 0.025, CI = 0.022-0.028. The study patients predominantly belonged 6^{th} and 7^{th} decade. The estrogen receptor positivty was more in sixth decade and eight decade.

AGE BASED OBSERVATION

CARCINOMA STOMACH



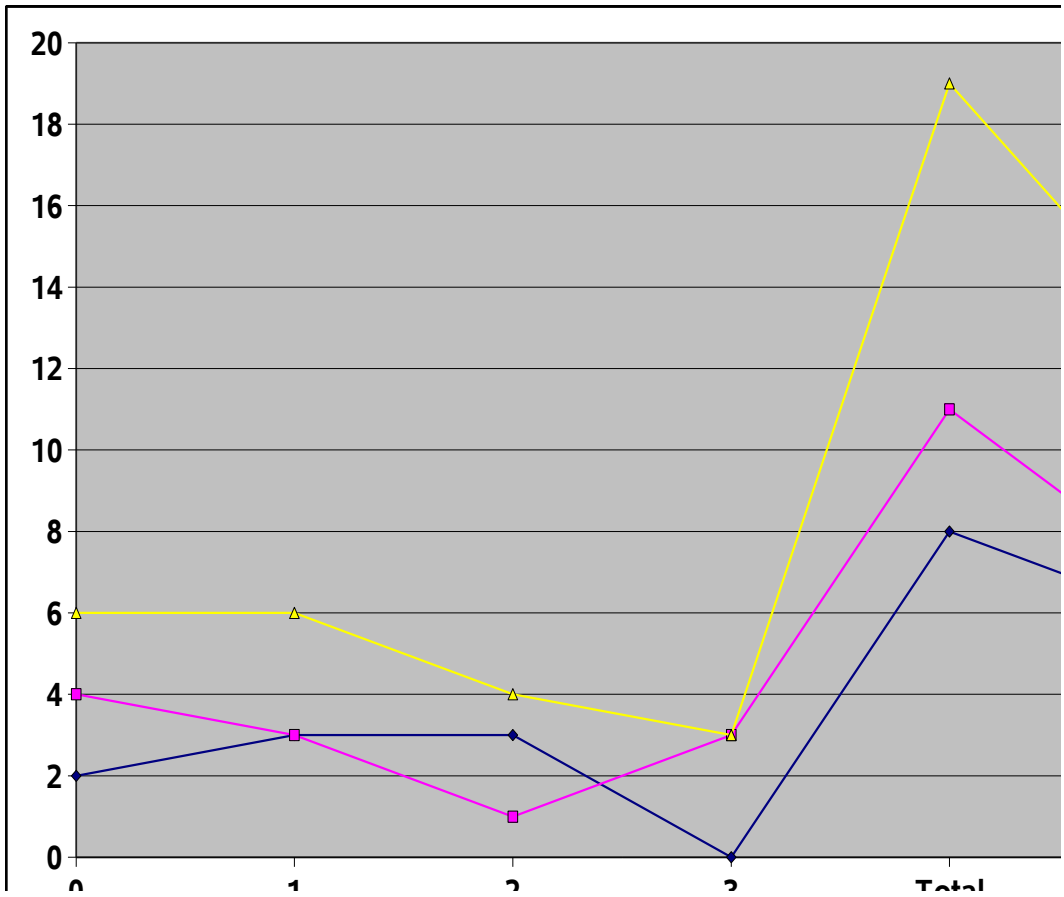
SEX BASED ESTROGEN RECEPTOR STATUS ESOPHAGEAL CARCINOMA

Sex	Intensity of Staining					
	0	**1+**	**2+**	**3+**	**Total patients**	**Total positivity**
Male	2	3	3	0	8	6
Female	4	3	1	3	11	7
Total	6	6	4	3	19	13

The above data was statistically analysed. χ^2 test = 0.45 df = 1, p.value= 1.000. The percentage of positivity of estrogen receptor had no difference among sexes in carcinoma esophagus.

SEX BASED DISTRIBUTION

CARCINOMA ESOPHAGUS



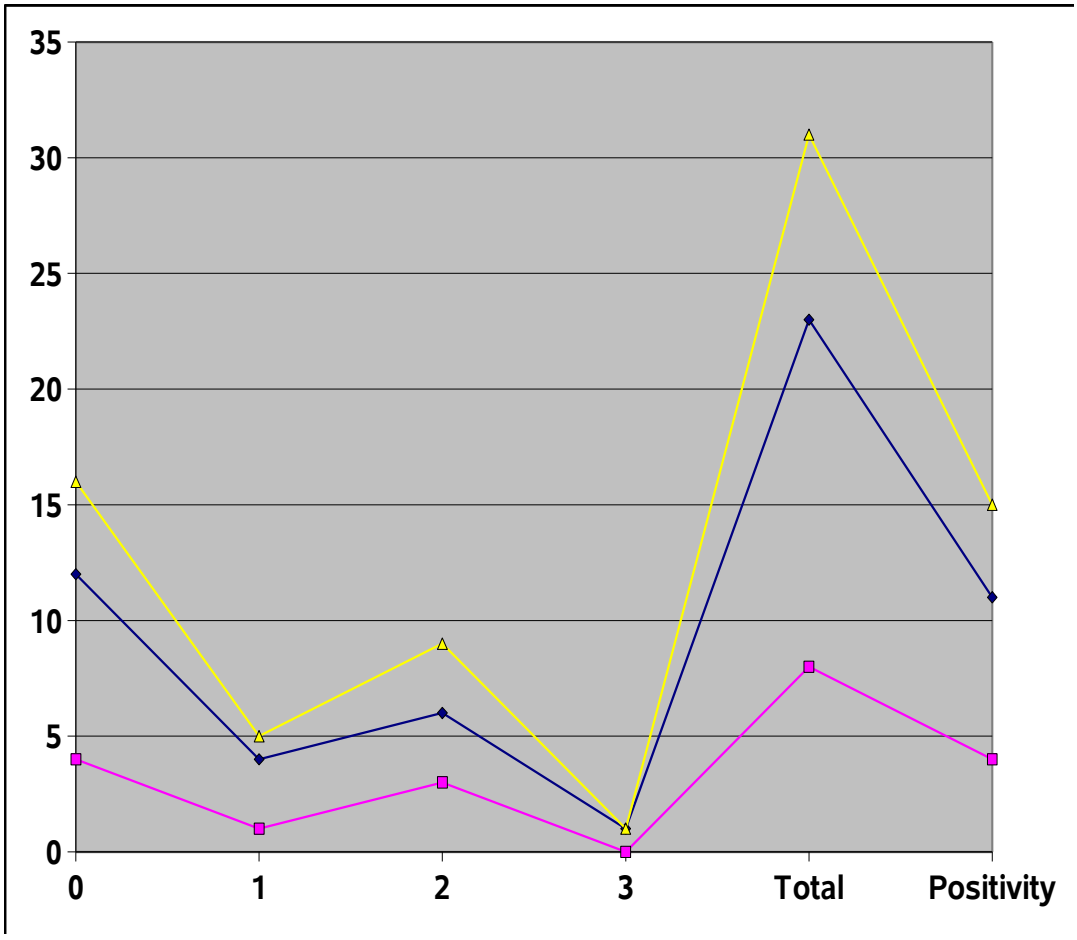
SEX BASED ESTROGEN RECEPTOR STATUS

STOMACH CARCINOMA

Sex	Intensity of Staining					
	0	**1+**	**2+**	**3+**	**Total patients**	**Total positivity**
Male	12	4	6	1	23	11
Female	4	1	3	0	8	4
Total	16	5	9	1	31	15

χ^2 test = 0.45, df = 1, p.value= 1.000. The percentage of estrogen receptor positivity is more in females when compared to males.

SEX BASED DISTRIBUTION
CARCINOMA STOMACH

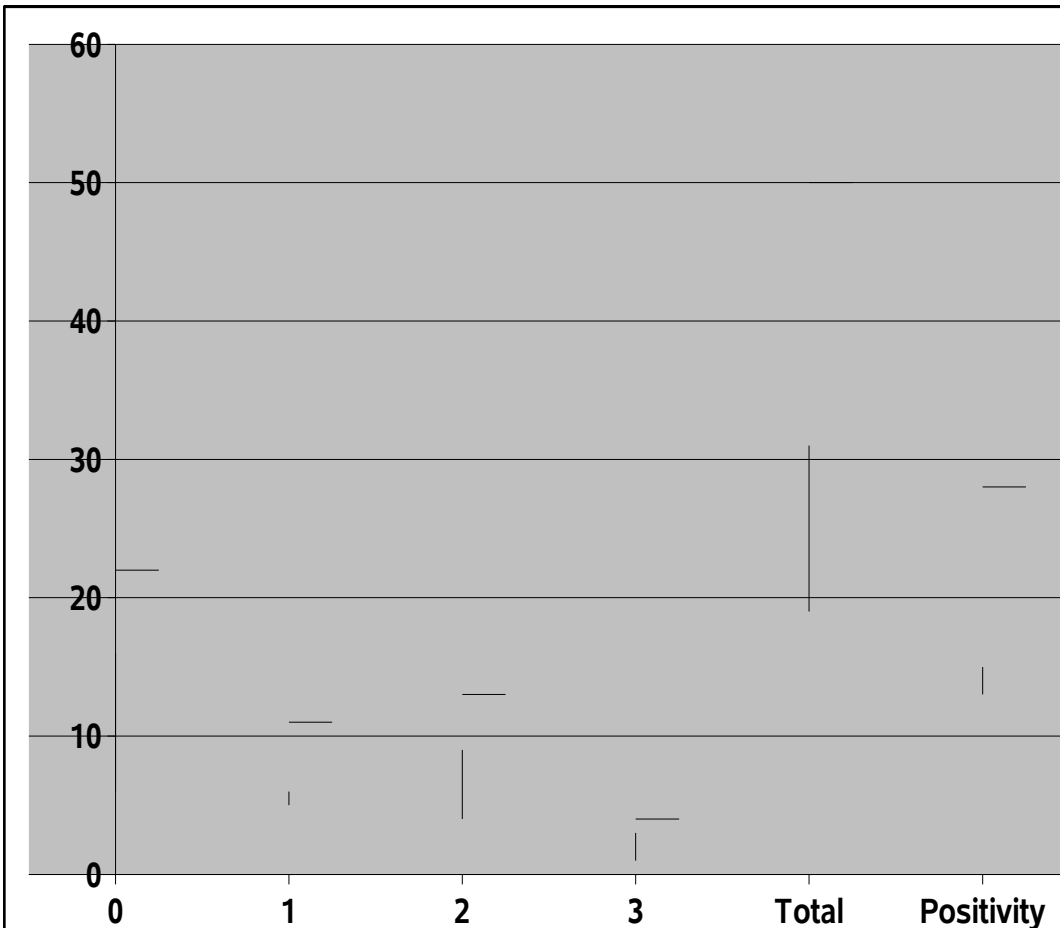


ORGAN BASED ESTROGEN RECEPTOR STATUS

Organ	Intensity of Staining					
	0	**1+**	**2+**	**3+**	**Total patients**	**Total positivity**
Esophagus	6	6	4	3	19	13
Stomach	16	5	9	1	31	15
Total	22	11	13	4	50	28

χ^2 test = 1.923, df = 2, p.value= 0.461, CI = 0.452-0.471. The percentage of estrogen receptor positivity is more in esophageal carcinoma when compared to stomach carcinoma.

ORGAN BASED DISTRIBUTION



HISTOPATHOLOGY BASED ESTROGEN RECEPTOR STATUS

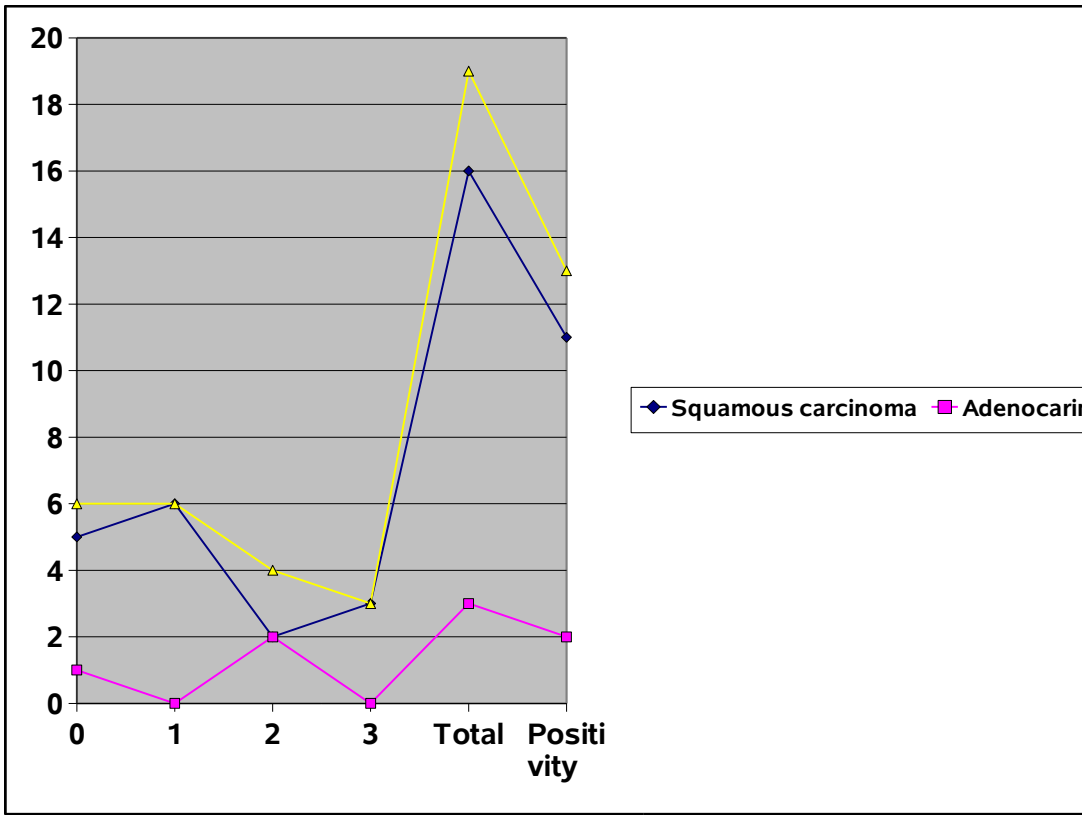
ESOPHAGEAL CARCINOMA

Carcinoma	**Intensity of Staining**					
	0	**1+**	**2+**	**3+**	**Total patients**	**Total positivity**
Squamous cell carcinoma	5	6	2	3	16	11
Adeno carcinoma	1	0	2	0	3	2
Total	6	6	4	3	19	13

The above data was statistically analysed. χ^2 test = 1.552, df =1, p.value= 0.240 The percentage of positivity for estrogen receptor, remains same for both histopathological types in carcinoma esophagus.

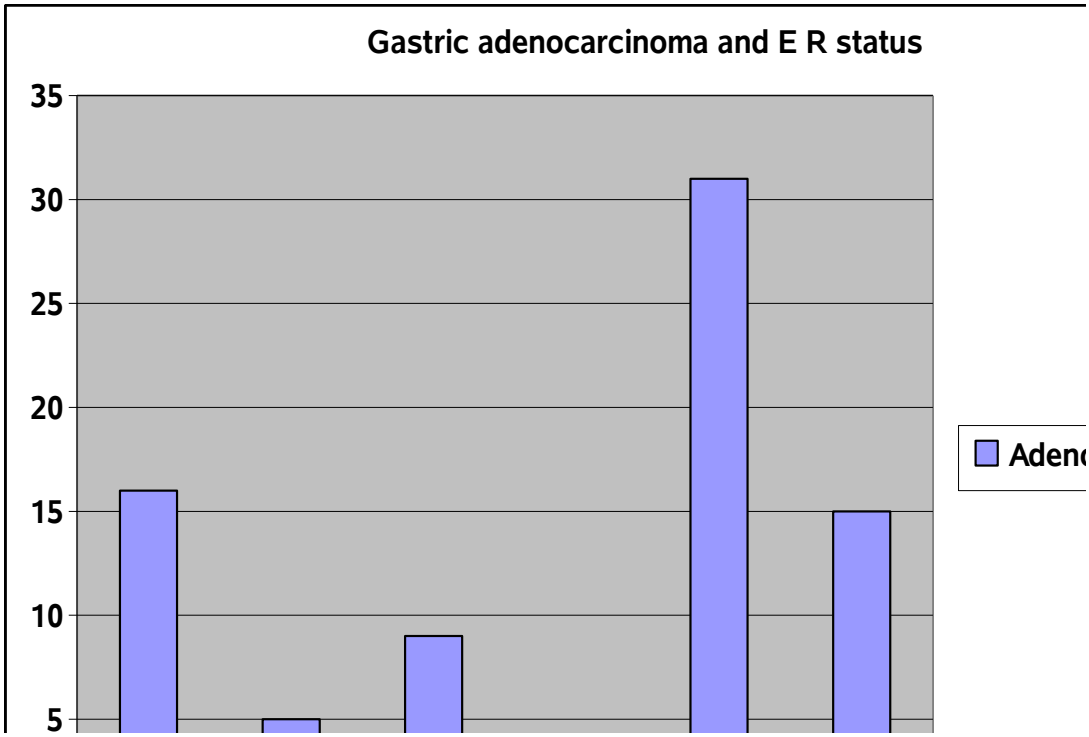
HISTOLOGY BASED ER STATUS

CARCINOMA ESOPHAGUS



GASTRIC CARCINOMA

Carcinoma	Intensity of Staining					
	0	1+	2+	3+	Total patients	Total positivity
Adeno carcinoma	16	5	9	1	31	15



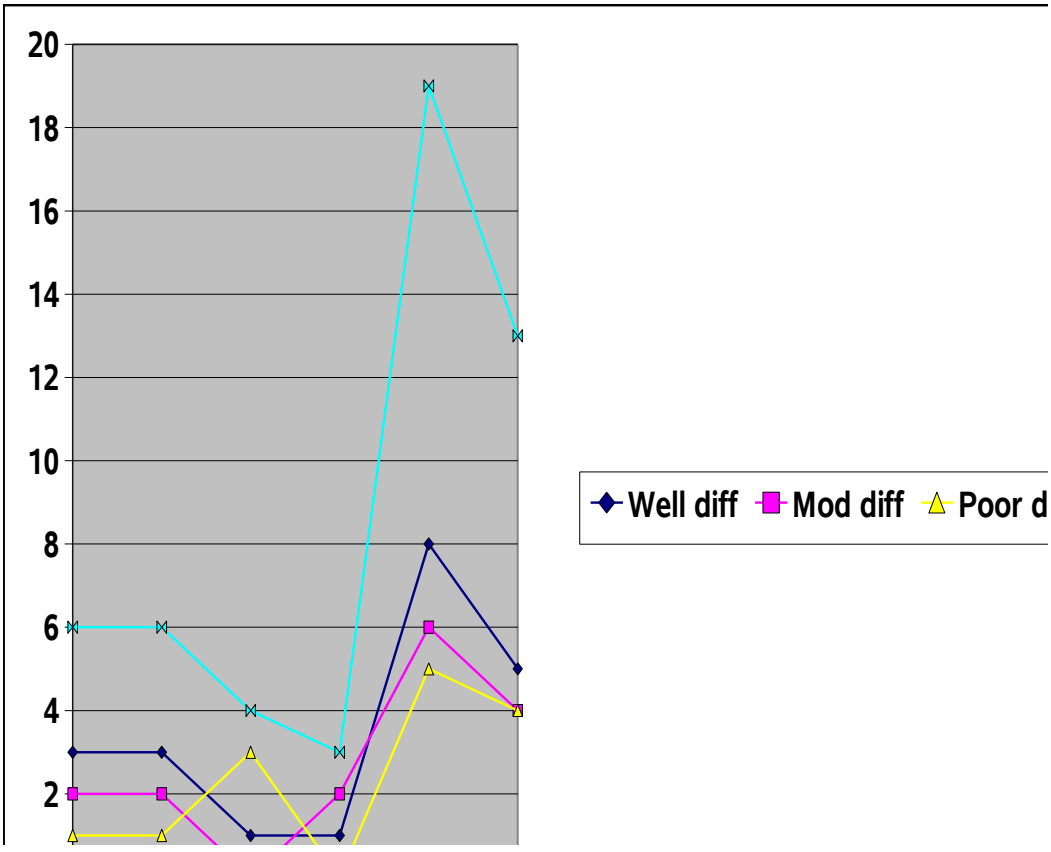
The above data was statistically analysed. χ^2 test = 1.552, df =1, p.value = 0.240. The estrogen receptor positivity is 48% in Gastric adenocarcinoma.

DIFFERENTIATION BASED ESTROGEN RECEPTOR STATUS ESOPHAGEAL CARCINOMA

Differentiation type	Intensity of Staining					
	0	**1+**	**2+**	**3+**	**Total patients**	**Total positivity**
Well differentiated	3	3	1	1	8	5
Moderately differentiated	2	2	0	2	6	4
Poorly differentiated	1	1	3	0	5	4
Total	6	6	4	3	19	13

The above data was statistically analysed. χ^2 test = 0.821 df=2, p.value= 0.748, CI = 0.739 - 0.756. Estrogen receptor positivity is more in poorly differentiated type than others.

DIFFERENTIATION BASED E R STATUS CARCINOMA ESOPHAGUS



DIFFERENTIATION BASED ER STATUS

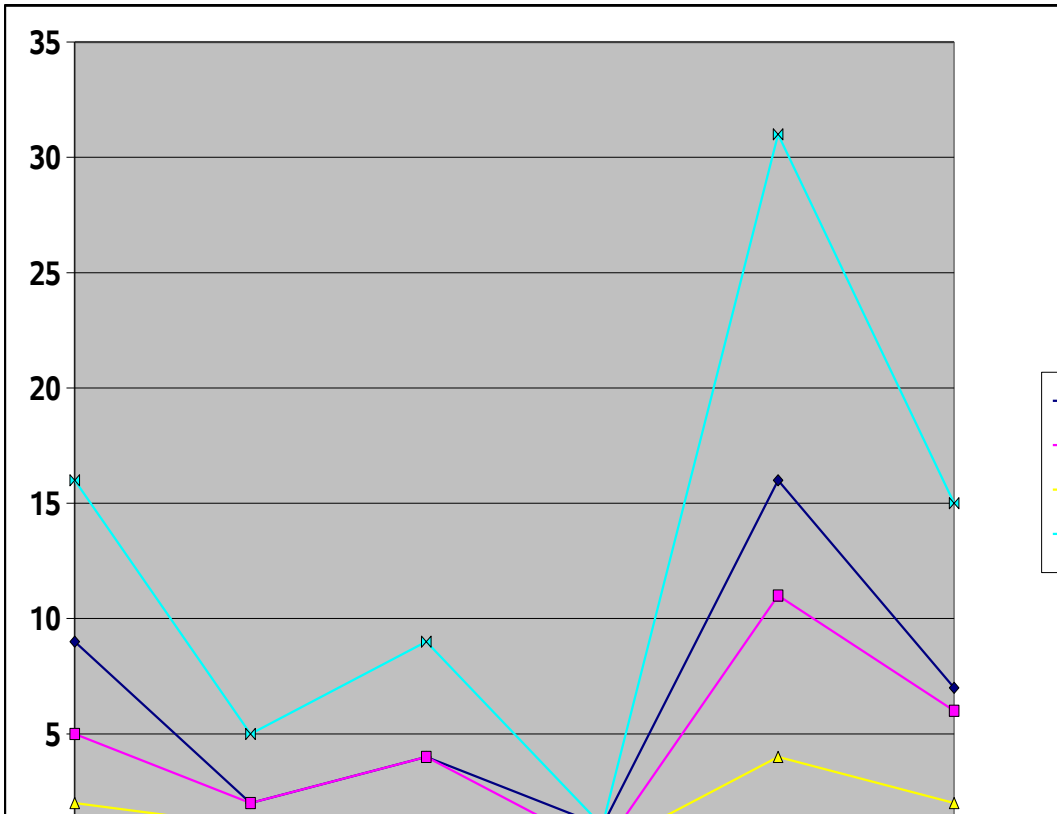
GASTRIC CARCINOMA

Differentiation type	Intensity of Staining					
	0	**1+**	**2+**	**3+**	**Total patients**	**Total positivity**
Well differentiated	9	2	4	1	16	7
Moderately differentiated	5	2	4	0	11	6
Poorly differentiated	2	1	1	0	4	2
Total	16	5	9		31	15

The above data was statistically analysed. χ^2 test = 0.821 df=2, p.value= 0.748, CI = 0.739 - 0.756. Estrogen receptor positivity is more in moderately differentiated type than others.

STOMACH ADENOCARCINOMA

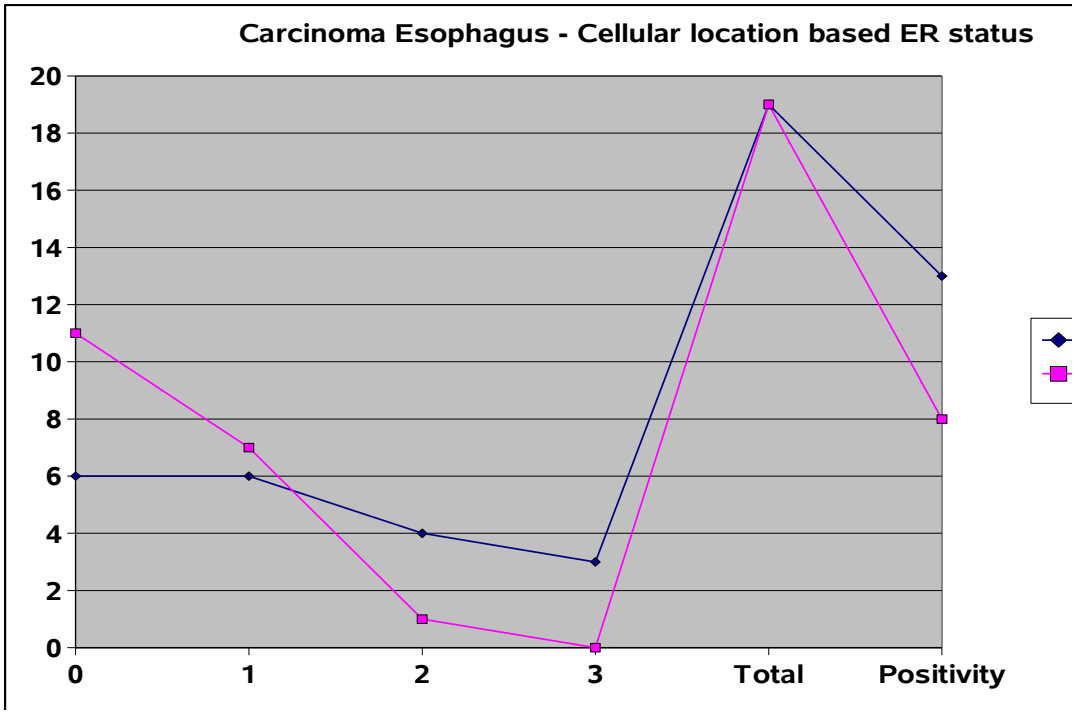
DIFFERENTIATION BASED E R STATUS



ESTROGEN RECEPTOR STATUS IN NUCLEUS AND CYTOPLASM

CARCINOMA ESOPHAGUS

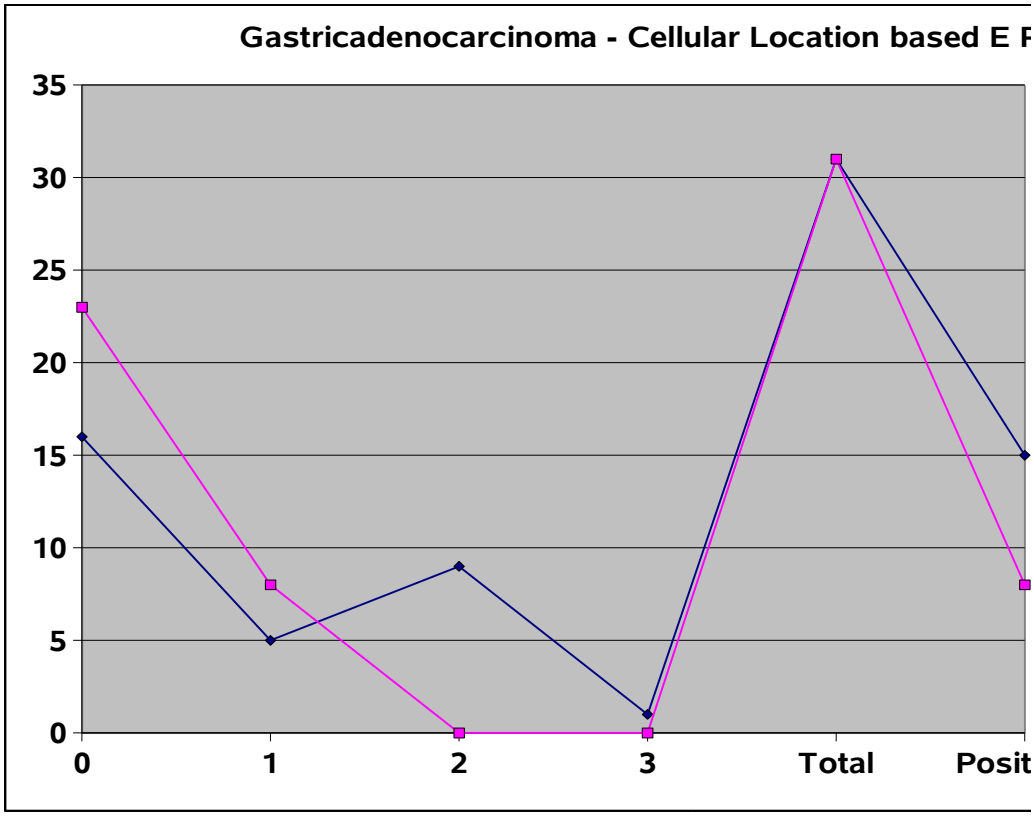
Location of positivity	Intensity of Staining					
	0	1+	2+	3+	Total patients	Total positivity
Cytoplasm	6	6	4	3	19	13
Nucleus	11	7	1	0	19	8



Estrogen receptor positivity is more in cytoplasm than in nucleus.

ESTROGEN RECEPTOR STATUS IN NUCLEUS AND CYTOPLASM CARCINOMA STOMACH

Location of positivity	Intensity of Staining					
	0	1+	2+	3+	Total patients	Total positivity
Cytoplasm	16	5	9	1	31	15
Nucleus	23	8	0	0	31	8



Estrogen receptor positivity is more in cytoplasm than in nucleus.

DISCUSSION

COMPARISON OF THIS STUDY WITH SIMILAR STUDIES

CARCINOMA STOMACH

Study Group	No. of Patients (n)	Estrogen Receptor Immunopositivity
Matsuyama et al (41)	29	100%
Zhao XH et al (19)	30	40%
Takano et al (43)	41	60%
Korenaga D et al (49)	23	56%
Oshima CT et al (14)	16	62.5%
Yokozaki H et al (50)	108	27.8%
Chu PG et al (51)	30	0%

The positive expression of Estrogen receptors in our Study in 48% and the total number of patients is 31 which is almost comparable with most of the Japanese study mentioned above. Some of the above studies having higher estrogen receptor positivity used Charcoal adsorption method than immunohistochemistry.

UNANSWERED QUESTIONS

1. Inspite of extensive molecular advances, no single mechanism or pathway has been solely implicated in any of the known human cancers. Hence with the present knowledge base available it is prudent for anyone to choose multiple drugs. This will ascertain the blockade of complex carcinogenetic pathway, is near complete to achieve maximal response. It remains to be known, whether isolated estrogen receptor modulation or a multimodal therapy is essential to show a definitive survival benefit.

2. Recently, the subtypes of estrogen receptors α and β are being found to be expressed in normal gastro intestinal tract and gastric cancers. The mRNA for both the subtypes has been detected in the gastric mucosa.. The cellular distribution of the estrogen receptor in various organelles determine the outcome, on the influence of estrogen. The distribution of estrogen receptor α and β are almost similar in fundic cells whereas in antrum β alone is better expressed. This indirectly suggests, estrogen inhibits gastric acid secretion via genomic effects in fundic parietal cells through either ER subtypes and antral cells via ER β. The expression of ER in enteric neurons indicate that estrogen effects could also be

mediated through neurogenic effects. This clearly implies that multiple cells expressing ER, contribute to the modulation of gastric function. Henceforth, now after detecting so much of ER positivity, it remains unanswered, whether achlorhydria caused by atrophic gastritis is indirectly controlled by ER. So the role of ER modulators in these type of premalignant conditions is yet to be quantified.

3. Work on the effect of 17 β estradiol and esophageal cancer cell lines have clearly shown, the increase in cell doubling time from 20 to 32 hrs by estrogen, when compared to untreated control. In reality, any tumour cell line cultured in vitro, has definitive less multifactorial influences when compared to malignancy in a living patient. The invitro to invivo translation of the above hypothesis will take a longer time, so the ultimate survival benefit, from estrogens in esophageal malignancy is yet to be known.

SUMMARY AND CONCLUSION

Estrogen receptor is a nuclear transcription factor, that is a member of the steroid receptor superfamily. Recent advances in the molecular biology, have helped us in evaluating the presence of estrogen receptor in upper gastro intestinal malignancies, indicating the role of hormones in therapeutic aspects.

We have done this study to identify the presence of estrogen receptor in upper gastrointestinal malignancies. The tissue obtained by endoscopic biopsy was utilised for this study. The histopathology was confirmed. The tumour tissue was subjected to immunohistochemistry examination for identification of estrogen receptor. Data obtained were based on various parameters.

In carcinoma esophagus out of the 19 patients studied 11 are females (55%) and 8 are males (45%). Patients with estrogen receptor positivity contributed to 68.42% and was more when compared to gastric malignancy. 90% of patients were between 41-80 years age group. Estrogen receptor positivity remained same in around 68% between adenocarcinoma and squamous cell carcinoma. Poorly differentiated cancer showed high estrogen receptor positivity (80%) when compared to other

differentiated types. Estrogen receptor positivity was more in the cytoplasm when compared to nuclear positivity

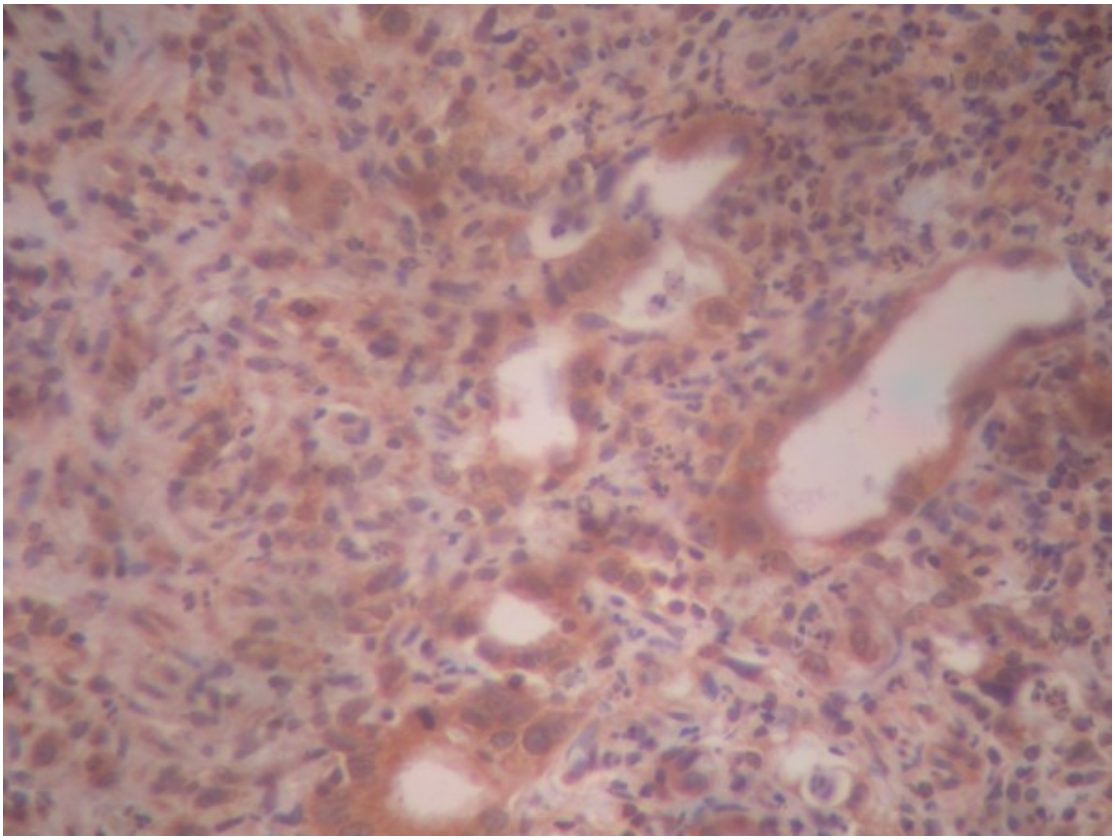
In carcinoma stomach out of 31 patients studied 23 were male (75%) and 8 were female (25%). Out of 31 patient, 15 were positive for estrogen receptor (48.38%). 90% of patients were between 41-80 years age group. Estrogen receptor positivity is 48% in gastric adenocarcinoma. Moderately differentiated type has higher estrogen receptor positivity (54.5%) when compared to other differentiated types (42-50%). Estrogen receptor positivity is more in cytoplasm when compared to nuclear positivity.

Identifying the estrogen receptor positivity will help in hormonal manipulation of the upper gastrointestinal malignancies. In future it may be of use in detecting premalignant conditions or early detection of cancerous transformation in high risk group. So it can possibly be used as a "screening tool".

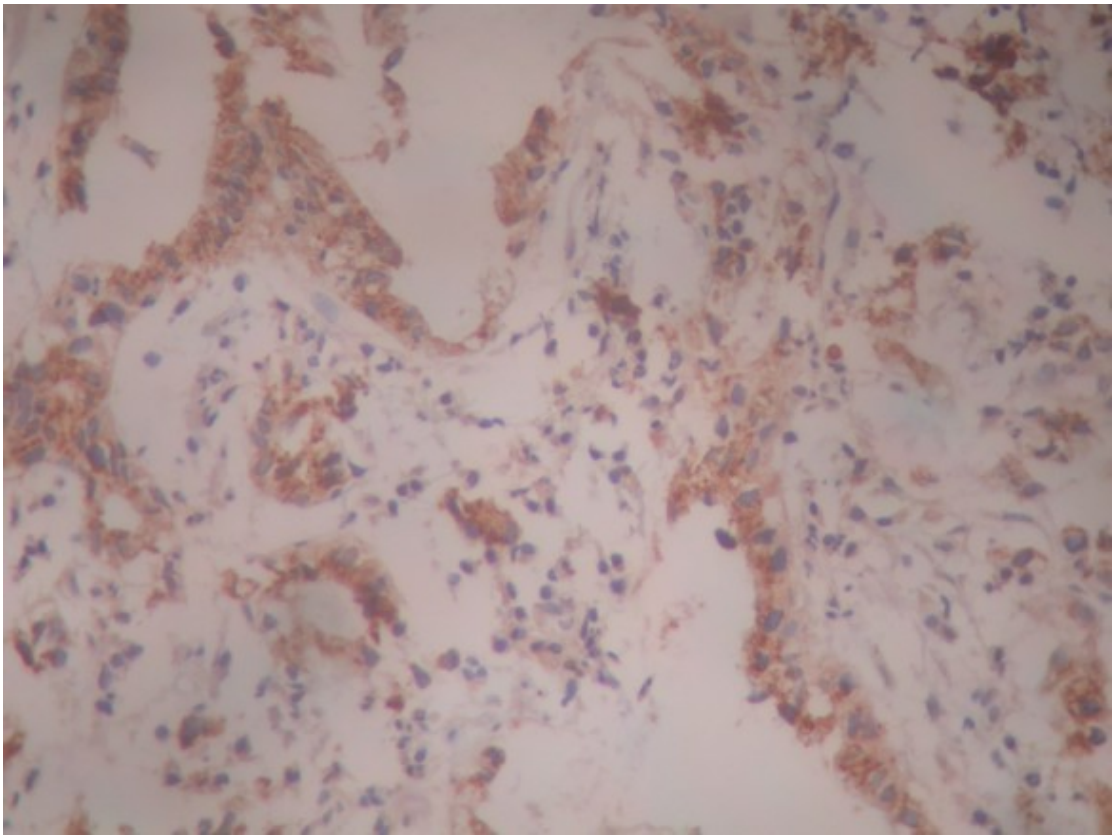
In advanced diseases, hormone therapy can be tried "to contain" the disease. We will be able to draw a firm conclusion from large volume studies on Indian population, with long time patients follow up.

PRIMARY ANTIBODY AND REAGENTS USED IN THIS STUDY

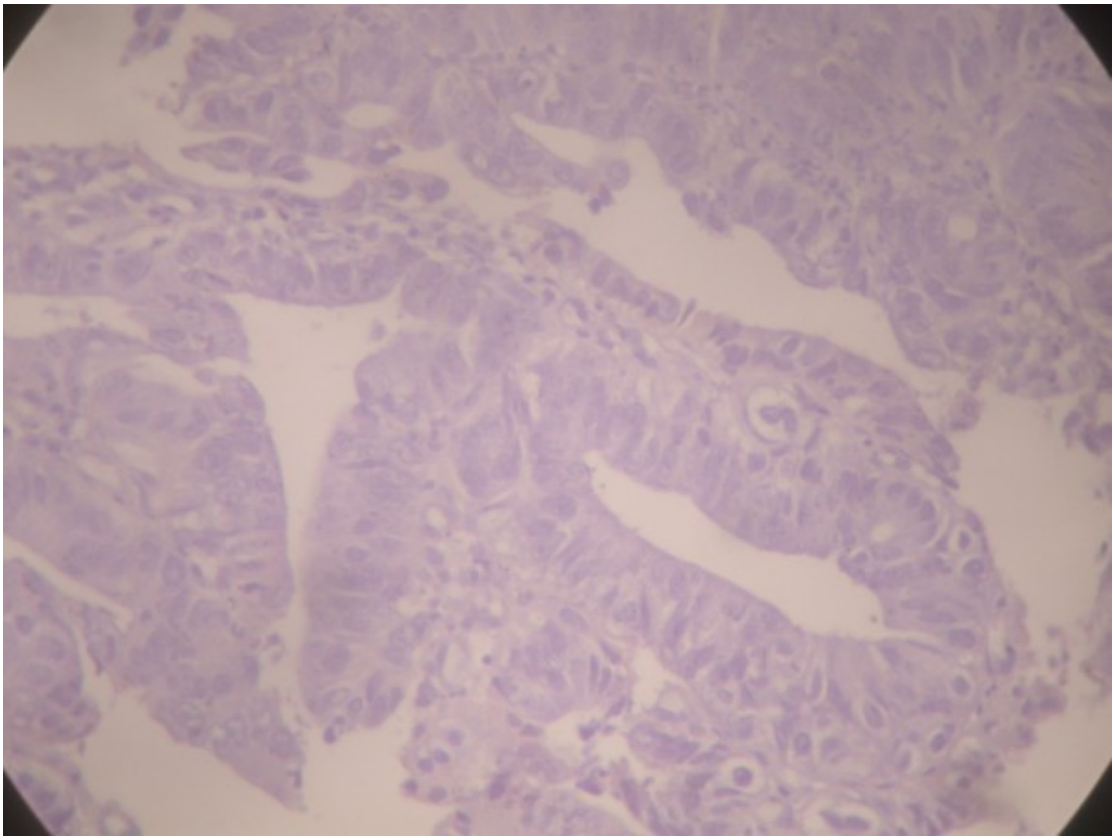
ESTROGEN RECEPTOR POSITIVE GASTRIC MALIGNANCY



ESTROGEN RECEPTOR POSITIVE ESOPHAGEAL MALIGNANCY



E S T R O G E N R E C E P T O R N E G A T I V E U G I M A L I G N A N C Y



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CUMULATIVE RESULTS

Sl. No	Patient Endoscopy I/D	Age	Sex	Site of Biopsy	Histopathology	Differentiation	Estrogen Receptor Status	Grading	
								Cytoplasm	Nucleus
1.	16/05	63	M	Esophagus	Squamous cell carcinoma	Moderately Differentiated	Positive	1+	1+
2.	22/05	47	F	Esophagus	Squamous cell carcinoma	Well Differentiated	Negative	-ve	-ve
3.	192/05	75	F	Esophagus	Squamous cell carcinoma	Well Differentiated	Positive	1+	-ve
4.	321/05	84	F	Esophagus	Squamous cell carcinoma	Well Differentiated	Positive	1+	-ve
5.	371/05	76	F	Esophagus	Squamous cell carcinoma	Well Differentiated	Positive	1+	1+
6.	24/05	67	M	Esophagus	Squamous cell carcinoma	Poorly Differentiated	Positive	2+	1+
7.	49/05	48	M	Esophagus	Squamous cell carcinoma	Moderately Differentiated	Positive	1+	-ve
8.	479/05	64	F	Esophagus	Squamous cell carcinoma	Well Differentiated	Negative	-ve	-ve
9.	05/05	63	F	Esophagus	Squamous cell carcinoma	Poorly Differentiated	Positive	2+	1+
10.	584/05	60	F	Esophagus	Squamous cell carcinoma	Moderately Differentiated	Positive	3+	2+
11.	606/05	52	F	Esophagus	Squamous cell carcinoma	Moderately Differentiated	Positive	3+	1+
12.	328/05	64	M	OG Junction	Squamous cell carcinoma	Moderately Differentiated	Negative	-ve	-ve
13.	610/05	65	F	Esophagus	Squamous cell carcinoma	Poorly Differentiated	Negative	-ve	-ve
14.	112/05	71	M	Esophagus	Squamous cell carcinoma	Poorly Differentiated	Positive	1+	-ve
15.	161/05	71	M	Esophagus	Squamous cell carcinoma	Moderately Differentiated	Negative	-ve	-ve

Sl. No	Patient Endoscopy I/D	Age	Sex	Site of Biopsy	Histopathology	Differentiation	Estrogen Receptor	Grading	
								Cytoplasm	Nucleus
16.	612/05	50	F	Esophagus	Squamous cell carcinoma	Well Differentiated	Positive	3+	1+
17.	358/05	60	M	OG Junction	Adenocarcinoma	Well Differentiated	Positive	2+	1+
18.	182/05	55	F	OG Junction	Adenocarcinoma	Well Differentiated	Negative	-ve	-ve
19.	253/05	75	M	OG Junction	Adenocarcinoma	Poorly Differentiated	Positive	2+	-ve
20.	104/05	68	F	Stomach	Adenocarcinoma	Moderately Differentiated	Positive	2+	1+
21.	39/05	52	M	Stomach	Adenocarcinoma	Poorly Differentiated	Positive	1+	1+
22.	56/05	83	M	Stomach	Adenocarcinoma	Well Differentiated	Positive	1+	-ve
23.	120/05	67	F	Stomach	Adenocarcinoma	Moderately Differentiated	Positive	2+	1+
24.	66/05	65	M	Stomach	Adenocarcinoma	Moderately Differentiated	Negative	-ve	-ve
25.	75/05	45	M	Stomach	Adenocarcinoma	Moderately Differentiated	Positive	2+	-ve
26.	180/05	59	F	Stomach	Adenocarcinoma	Well Differentiated	Positive	2+	1+
27.	220/05	37	F	Stomach	Adenocarcinoma	Well Differentiated	Negative	-ve	-ve
28.	111/05	73	M	Stomach	Adenocarcinoma	Well Differentiated	Positive	2+	-ve
29.	231/05	64	M	Stomach	Adenocarcinoma	Moderately Differentiated	Negative	-ve	-ve
30.	295/05	52	M	Stomach	Adenocarcinoma	Moderately Differentiated	Positive	2+	-ve
31.	375/05	55	M	Stomach	Adenocarcinoma	Well Differentiated	Negative	-ve	-ve
32.	347/05	41	M	Stomach	Adenocarcinoma	Moderately Differentiated	Positive	1+	-ve
33.	277/05	75	F	Stomach	Adenocarcinoma	Poorly Differentiated	Negative	-ve	-ve
34.	284/05	58	F	Stomach	Adenocarcinoma	Well Differentiated	Negative	-ve	-ve
35.	380/05	50	M	Stomach	Adenocarcinoma	Well Differentiated	Negative	-ve	-ve
36.	404/05	75	M	Stomach	Adenocarcinoma	Well Differentiated	Negative	-ve	-ve
37.	442/05	42	M	Stomach	Adenocarcinoma	Moderately Differentiated	Negative	-ve	-ve
38.	467/05	51	M	Stomach	Adenocarcinoma	Moderately Differentiated	Positive	1+	1+
39.	388/05	76	F	Stomach	Adenocarcinoma	Well Differentiated	Positive	1+	-ve

Sl. No	Patient Endoscopy I/D	Age	Sex	Site of Biopsy	Histopathology	Differentiation	Estrogen Receptor	Grading	
								Cytoplasm	Nucleus
40.	470/05	79	M	Stomach	Adenocarcinoma	Well Differentiated	Positive	2+	1+
41.	472/05	50	M	Stomach	Adenocarcinoma	Well Differentiated	Positive	3+	1+
42.	481/05	47	M	Stomach	Adenocarcinoma	Well Differentiated	Negative	-ve	-ve
43.	487/05	76	M	Stomach	Adenocarcinoma	Poorly Differentiated	Positive	2+	-ve
44.	499/05	59	M	Stomach	Adenocarcinoma	Well Differentiated	Negative	-ve	-ve
45.	480/05	58	F	Stomach	Adenocarcinoma	Moderately Differentiated	Negative	-ve	-ve
46.	504/05	58	M	Stomach	Adenocarcinoma	Well Differentiated	Positive	2+	1+
47.	509/05	69	M	Stomach	Adenocarcinoma	Moderately Differentiated	Negative	-ve	-ve
48.	521/05	65	M	Stomach	Adenocarcinoma	Well Differentiated	Negative	-ve	-ve
49.	531/05	45	M	Stomach	Adenocarcinoma	Poorly Differentiated	Negative	-ve	-ve
50.	545/05	65	M	Stomach	Adenocarcinoma	Well Differentiated	Negative	-ve	-ve

STUDY FORMAT

Name :

Age :

Sex :

a. Male

b. Female

Patient Endoscopy ID No.

Location of the tumor

a. Esophagus

b. OG Junction

c. Stomach

Histopathology of the tumor

Adenocarcinoma

Squamous cell Carcinoma

Degree of Differentiation

a. Well differentiated

b. Moderately differentiated

c. Poorly differentiated

Immunohistochemistry

a. Positive

b. Negative

Degree of Staining

a. Grade 1+

b. Grade 2+

b. Grade 3+